lovE Variant Regulator Molecules
(Atty Docket No. 109272.150; Client Docket No. MIC005US)

BACKGROUND OF THE INVENTION

Field of the Invention

The invention relates to the fields of microbiology and molecular biology. In particular, the invention relates to the field of mycology and the production of secondary metabolites from fungi.

Summary of the Related Art

Secondary metabolites are a major source of commercially useful products such as food additives, vitamins, and medicines for the treatment of a wide variety of infections and diseases. By way of example, in 1997 the statin drugs lovastatin, simvastatin, and pravastatin, fungal secondary metabolites used in the treatment of hypercholesteremia, together had US sales of US$7.53 billion (Sutherland *et al.*, *Current Opinion In Drug Discovery & Development* **4**:229-236 (2001)). The cost and availability of these plant, bacterial and fungal metabolites are frequently determined by limitations imposed on production and purification of these compounds from culture. This problem is frequently exacerbated by the fact that these products are generally produced during the stationary phase of bacterial and fungal growth.

A wide variety of methods have been utilized to increase the amount of secondary metabolite produced in culture. Studies have demonstrated the importance of carefully designing the medium in which a fungus is grown to maximize the amount of a secondary metabolite produced (see, *e.g.*, Hajjaj H, *et al.*, *Appl. Environ. Microbiol.* **67**:2596-602 (2001); Lesova, K., *et al.*, *J. Basic Microbiol.* **40**:369-75 (2000)). In addition, the method of culture or fermentation also impacts directly on the amount of secondary metabolite produced. For example, see Robinson, T., *et al.* (*Appl. Microbiol. Biotechnol.* **55**:284-

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289 (2001)), which demonstrates the advantages of solid state (substrate) fermentation.

In addition to the manipulation of culture and media conditions, genetic approaches have been taken to increase secondary metabolite production. For example, the production of penicillin is limited by the activity of two enzymes, encoded by the *ipnA* and *acvA* genes, both of which are regulated by the *pacC* protein, a zinc-finger transcription factor. Naturally occurring mutant alleles of the *pacC* locus are known to possess more transcription-activating activity than the cognate, wild-type allele (see, *e.g.*, Tilburn *et al. EMBO J.* **14(4)**:779-790 (1995)). Thus, one genetic approach to increasing secondary metabolite production is to identify and isolate naturally occurring mutant alleles, the expression of which leads to increased secondary metabolite production.

Although many regulators of secondary metabolite production in many organisms are known, not all of the organisms that produce secondary metabolites are amenable to genetic or molecular genetic manipulation. Thus, these systems are not generally useful as a source for the isolation of naturally occurring mutant alleles and are even less useful for the deliberate manipulation of secondary metabolite regulator protein structure with the aim of creating improved regulators of secondary metabolite production.

It would be advantageous to have improved regulators of the biosynthetic enzymes responsible for secondary metabolite production. For example, recent studies suggest increasing usage of statin drugs, e.g., see Waters D.D., *Am. J. Cardiol.* **88**:10F-5F (2001)). Thus, demand for statin drugs is likely to increase substantially. In order to meet the demand for these and other secondary metabolites, new and improved methods for the production of secondary metabolites must be identified.

BRIEF SUMMARY OF THE INVENTION

The invention provides improved secondary metabolite regulator proteins that enable increased production of secondary metabolites. The invention also provides methods to make these improved regulator proteins.

In a first aspect, the invention provides a variant regulator protein of secondary metabolite production with increased activity than that of the cognate, wild-type protein. In certain embodiments of this aspect of the invention, the regulator protein is a fungal regulator protein.

In an embodiment of the first aspect, the invention provides an improved regulator protein comprising an amino acid sequence coding for a variant lovE protein having at least one specific mutation that gives rise to greater transcription-activating properties of the regulator protein and/or induction of secondary metabolite synthesis.

By way of non-limiting example, certain preferred regulator proteins of this aspect of the invention include at least one of the following mutations: (1) a Group 6 amino acid residue mutated to a Group 2 amino acid residue at position 31, in one embodiment the mutation represented by F31L; (2) a Group 3 amino acid residue mutated to a Group 5 amino acid residue at position 41, in one embodiment the mutation represented by Q41K or Q41R; (3) a Group 4 amino acid residue mutated to a Group 2 amino acid residue at position 52, in one embodiment the mutation represented by T52I; (4) a Group 4 amino acid residue mutated to a Group 3 amino acid residue at position 52, in one embodiment the mutation represented by T52N; (5) a Group 4 amino acid residue mutated to a Group 5 amino acid residue at position 73, in one embodiment the mutation represented by C73R; (6) a Group 1 amino acid residue mutated to a Group 4 amino acid residue at position 101, in one embodiment the mutation represented by P101S; (7) a Group 1 amino acid residue mutated to a Group 3 amino acid residue at position 101, in one embodiment the mutation